



Attorney's Docket No.: 029996/0278721

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Kieffer et al. Art Unit : 1632
Serial No. : 09/804,409 Examiner : Paras, P.
Filed : 03/12/01
Title : COMPOSITIONS AND METHODS FOR REGULATED PROTEIN
EXPRESSION IN GUT

Assistant Commissioner of Patents
Washington, DC 20231

DECLARATION OF DR. ANTHONY CHEUNG
UNDER 37 C.F.R. §1.132

Sir:

1. I, TIMOTHY KIEFFER, declare and say I am a resident of Vancouver, Canada. My residence address is: 4284 W 15th Ave.
2. I received Bachelor of Science degree in from the University of British Columbia, in 1989. I received a Doctor of Philosophy degree in Physiology from the University of British Columbia in 1994. I am currently Vice President, Research and Development of Engene, Inc. My curriculum vitae is attached, which reflects my expertise in the areas of biochemistry and molecular biology, gene delivery systems and animal protein expression systems.
3. I am an inventor of the subject matter claimed in United States Patent Application Serial No. 09/804,409, filed March 12, 2001.
4. I have read the Office Action and understand that the Examiner questions that the methods are adequately enabled for producing a therapeutic protein *in vivo* for treating or preventing a disease.
5. I submit this declaration to provide corroborating evidence that, as disclosed in the specification, a therapeutic protein can be produced *in vivo* in amounts effective to treat a disorder as is claimed.

Attorney's Docket No.: 029996/0278721

6. The following studies, performed by me or under my direction, employed compositions and techniques disclosed in the specification. In particular, other therapeutic polypeptides including leptin are disclosed, for example, at page 20, lines 13-14, and page 23, lines 1-4; mucosal cells including Gut K cells and STC-1 cells are disclosed, for example, at page 25, lines 6-7 and lines 14-17, at page 28, lines 20-25, and at page 43, lines 19-22; and *in vitro*, *in vivo* and *ex vivo* delivery methods are disclosed, for example, at page 37, line 19, through page 38, line 29.
7. Gut K-cells are enteroendocrine cells located in the proximal small intestine and the stomach having secretory capabilities. This secretory capability provides an increase in therapeutic peptides such as insulin or leptin secretion in response to a meal.
8. GTC-1 cells are Gut K-cells derived from an intestinal tumor cell line, STC-1, as disclosed in the specification (see, for example, page 43, line 19, to page 44, line 11). GTC-1 cells were genetically modified to produce leptin and engineered to contain a regulatory switch system. The inducible regulatory system employed was a modified GeneSwitch System (Wang et al. Proc Natl Acad Sci USA 91:8180-4 (1994)).
9. In brief, the modified GeneSwitch System employs transcription factors composed of functional domains (DNA binding domains, activation domains) contained within a hybrid regulatory GAL4 protein. This hybrid regulatory GAL4 protein binds to an inducible agent, mifepristone (RU486), and functions as a ligand-dependent transcription factor that induces expression of the leptin gene. The regulatory plasmid, pSwitch, encodes this hybrid Gal4 regulatory protein, which is under the control of the glucose-dependent insulinotropic polypeptide promoter (GIPro; see, for example, the specification at page 44, lines 12-21). This modified pSwitch plasmid was generated by removing the GAL4 upstream activating sequence (UAS)/*PTK* promoter region from the pSwitch vector and replacing it with GIPro (GIPro was obtained from pGIPro-FE by digestion with restriction enzymes and into the pSwitch vector), thereby producing the pSwitchGIPro plasmid. The responsive expression plasmid, pGeneB/hLeptin/(-)V5His, expresses the human leptin gene (hLeptin gene derived from Human Fat Cell) under the control of a hybrid promoter containing Gal4 upstream activating sequences (UAS). These 2 plasmids were transfected into GTC-1 cells, providing regulatory control of

Attorney's Docket No.: 029996/0278721

leptin expression by RU486 (GTC-1pSwitchGIPro/hLeptin cells, hereafter denoted GTC-1 pSwitch cells).

10. In the transfected GTC-1 cells (GTC-1pSwitch), RU486 stimulates transcription factor production and, in turn, transcription of the leptin transgene. As described below, as the amount of RU486 increases the amount of leptin produced and secretion also increases, providing RU486 dose-dependent regulation of leptin secretion.
11. In vitro Studies: Transfected GTC-1pSwitch cells were induced with various doses of RU486 (1×10^{-6} M RU486, 1×10^{-8} M RU486, 1×10^{-9} M RU486, 1×10^{-10} M RU486, 1×10^{-11} M RU486, 1×10^{-12} M RU486) and incubated for 24 hours; as well, these GTC-1pSwitch cells were induced with 1×10^{-8} M RU486 and incubated for various time points (4 hours, 8 hours, 24 hours, 32 hours, 48 hours, 56 hours and 72 hours). Samples of cell culture medium were collected and Northern, Western and ELISA analysis performed.
12. Northern blot analysis revealed that human leptin mRNA was not detected without RU486. As the amount of RU486 increased, the amount of leptin mRNA also increased, reaching a maximal level at 1×10^{-6} M RU486 (see Figures 1-4, attached herewith). Specifically, as the amount of time the GTC-1pSwitch cells were incubated with RU486 increased, the amount of human leptin mRNA also increased. Northern blot analysis also revealed that human leptin mRNA was absent at 0 hours. This data indicates that RU486 stimulates leptin transcription, and that the amount of leptin mRNA is dose and time dependent.
13. Western blot analysis confirmed that the amount of leptin produced is dose and time dependent, and that RU486 is necessary before leptin is detected (Figures 5-8, attached herewith). Samples of culture medium analyzed for leptin with Enzyme-Linked Immunosorbent Assay (ELISA) further confirmed that the amount of leptin secreted by the GTC-1pSwitch cells is dose and time dependent, and that leptin is only secreted in the presence of RU486 (see Figure 9, attached herewith).
14. The *in vitro* data therefore indicate that the leptin secreted by the GTC-1pSwitch cells is dependent upon RU486 and, furthermore, that the amount of leptin produced is dose and time dependent.

Attorney's Docket No.: 029996/0278721

15. *In vivo Studies*: Ob/ob mice were transplanted with GTC-1pSwitch cells that were encapsulated in sodium alginate by passing a alginate/cell suspension through an electrostatic field interaction coupled with syringe pump extrusion. Approximately 1 million encapsulated cells were transplanted into the peritoneal space through a 20-gauge catheter in each animal after 49 days of tracking body weight and blood glucose levels; RU486 pellets containing either 0.875 µg, 8.75 µg, or 87.5 µg RU486 were implanted subcutaneously at the same time. Control mice received GTC-1 cells engineered to produce the switch protein, but not leptin, along with 87.5 µg RU486 pellets. The ob/ob mice were maintained over a 100-day time period and changes in body weight and blood glucose concentrations monitored (Figures 10 and 11; data points are the mean values for 4 mice per group).
16. The *in vivo* data indicate that the ob/ob mice lost weight during the time that RU486 was administered. Cessation of RU486 release from the pellet after 2 weeks was associated with a rapid increase in weight gain.
17. Blood glucose concentrations rose to diabetic levels in the animals prior to cell transplant and were normalized for the duration of RU486 release. Furthermore, normalized values persisted for days after the cessation of RU486 release. Moreover, the ob/ob mice maintained normal blood glucose concentrations subsequent to depletion of the 2-week RU486 pellets, even though their body weight rose back to pre-treatment levels at which they were previously diabetic.
18. This *in vivo* data therefore indicate that leptin secreted by the implanted GTC-1pSwitch cells reduce obesity in mice. The *in vivo* data further indicate that the secreted leptin normalized blood glucose concentrations in the animals, even after withdrawal of the leptin inducer RU486.
19. The studies described above corroborate that, as disclosed in the specification, an amount of a therapeutic protein can be produced *in vivo* that is effective at treating a disorder.
20. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title

Attorney's Docket No.: 029996/0278721

XVIII of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

June 18, 2003
Date

T. Kieffer
TIMOTHY KIEFFER, Ph.D.



Timothy James Kieffer, Ph.D.

CURRICULUM VITAE

PERSONAL DATA

NAME: Timothy James Kieffer

HOME ADDRESS: 4284 West 15th Ave, Vancouver, BC V6R 3A6

WORK ADDRESS: 2146 Health Sciences Mall, UBC, Vancouver, BC V6T 1Z3

MARITAL STATUS: Married; Stephanie Anne Kieffer

BIRTH DATE: March 14, 1966

PLACE OF BIRTH: Sebastopol, California; Canadian/American Citizenship

EDUCATION AND TRAINING

Education:

1989	B.Sc.	University of British Columbia
1994	Ph.D.	University of British Columbia

Postdoctoral Training:

1994 - 1996	Postdoctoral Fellow	Massachusetts General Hospital Harvard Medical School
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HONORS AND AWARDS

1998	Scholarship	Canadian Diabetes Association
1998	Scholarship	Alberta Heritage Foundation for Medical Research
2001	Career Development Award	Juvenile Diabetes Research Foundation

PROFESSIONAL/ACADEMIC POSITIONS HELD

Academic Appointments:

1997 - 1998	Instructor of Medicine	Harvard Medical School
1998 - 2001	Assistant Professor of Medicine	University of Alberta
2002 - 2002	Associate Professor of Medicine	University of Alberta
2002 - present	Associate Professor of Medicine	University of British Columbia

Hospital Appointments:

1997 - 1998	Assistant in Medicine	Massachusetts General Hospital
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Other Professional Appointments:

1994 - 1998	Research Associate	Howard Hughes Medical Institute
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RESEARCH

Recent Invited Presentations:

- 1996 11th International Symposium on Regulatory Peptides, Copenhagen, Denmark.
- 1996 24th New England Endocrine Conference, Dartmouth College, Hanover NH, USA.
- 1997 Department of Medicine, University of Alberta, Edmonton AB, Canada.
- 1997 Boston-Ithaca Islet Club Meeting, Woods Hole MA, USA.
- 1997 Endocrine Division, Massachusetts General Hospital, Boston MA, USA.
- 1998 41st Canadian Federation of Biological Societies, Edmonton AB, Canada.
- 1998 Department of Physiology, University of Alberta, Edmonton AB, Canada.
- 1998 Muttart Diabetes Research and Training Centre, Edmonton AB, Canada.
- 1999 Canadian Diabetes Association Professional Conference, Ottawa ON, Canada.
- 2000 Collip Club, University of Alberta, Edmonton AB, Canada.
- 2000 Department of Medicine, University of Alberta, Edmonton AB, Canada.
- 2000 Department of Physiology, University of British Columbia, BC, Canada.
- 2000 City-Wide Endocrine Rounds, Mount Sinai Hospital, Toronto ON, Canada.
- 2001 Medical Grand Rounds, University of Alberta, Edmonton AB, Canada.
- 2001 Department of Developmental Biology, Hagedorn Research Institute, Denmark.
- 2001 Dept. of Metab, Endo. & Molecular Medicine, University of Wurzburg, Germany.
- 2001 11st Scientific Sessions, American Diabetes Association, Philadelphia PA, USA.
- 2001 AHFMR & UA Faculty of Medicine Public Lecture, Edmonton AB, Canada.
- 2001 BC Research Institute for Children's & Women's Health, Vancouver BC, Canada.
- 2001 NIDDK Workshop: Beta Cell Biology in the 21st Century, Bethesda MD, USA.
- 2001 Focus on: Diabetes, 6th International Meeting, Erice Italy.
- 2002 Department of Physiology, University of British Columbia, BC, Canada.
- 2002 Stem Cell Network Workshop, Montreal Quebec, Canada.
- 2002 JDRF Kids and Us Public Conference, Edmonton Alberta, Canada.
- 2002 German Diabetes Association Conference, Dresden Germany.

Regular Reviewing Activities:

Journals – Diabetes, Diabetologia, Endocrinology, Am. J. Physiol., J. Clin. Endo.

Metab., Trends in Endo. Metab., Trends in Molec. Med., Regulatory Peptides

Agencies – Canadian Institutes of Health Research, Canadian Diabetes Association, Heart and Stroke Foundation



Grant Support (Since 1998):

1998 - 1999	Alberta Heritage Foundation for Medical Research	\$190,000
	Topic: <i>Leptin regulation of islet function/glucose homeostasis.</i>	
1998 - 1999	Intellectual Infrastructure Partnership Program	\$119,845
	Topic: <i>Laboratory of molecular endocrinology (establishment).</i>	
1998 - 1999	Canadian Diabetes Association	\$10,000
	Topic: <i>Leptin regulation of islet function/glucose homeostasis.</i>	
1999 - 2000	Alberta Heritage Foundation for Medical Research	\$35,000
	Topic: <i>Phase 1 technology commercialization program.</i>	
1999 - 2001	Canadian Diabetes Association	\$120,000
	Topic: <i>Effect of a GIP antagonist on glucose tolerance.</i>	
2001 - 2003	Canadian Diabetes Association	\$134,788
	Topic: <i>Effect of a GIP antagonist on glucose tolerance.</i>	
1998 - 2001	Medical Research Council of Canada	\$188,355
	Topic: <i>Leptin regulation of beta-cell function.</i>	
2001 - 2005	Canadian Institutes of Health Research	\$193,580
	Topic: <i>Leptin regulation of beta-cell function.</i>	
2000 - 2003	Juvenile Diabetes Research Foundation	\$430,000
	Topic: <i>Targeted expression of insulin to intestinal endocrine cells.</i>	
2001 - 2006	Juvenile Diabetes Research Foundation	\$896,610
	Topic: <i>Career Development Award.</i>	
2001 - 2005	Networks of Centres of Excellence (Co-PI)	\$21,124,000
	Topic: <i>The Stem Cell Genomics and Therapeutics Network</i>	
2002 - 2007	CIHR New Emerging Team (Co-PI)	\$1,230,000
	Topic: <i>Pancreatic Islet Generation from Human Stem Cells</i>	

BIBLIOGRAPHY

Publications:

1. Kieffer T.J., Verchere C.B., Fell C.D., Huang Z., Brown J.C., and Pederson R.A. Glucose-dependent insulintropic polypeptide stimulated insulin release from a tumor-derived β -cell line (β TC3). *Can. J. Physiol. Pharmacol.* 71:917-922 (1993).
2. Kieffer T.J., Buchan A.M.J., Barker H., Brown J.C., and Pederson R.A. Release of gastric inhibitory polypeptide (GIP) from cultured canine endocrine cells. *Am. J. Physiol.* 267:E489-E496 (1994).
3. Kieffer T.J., Schieldrop P.J., McLean E., Donaldson E.M., and Brown J.C. A radioimmunoassay for Oncorhynchid growth hormone targeted to the physiological range. *Can. J. Physiol. Pharmacol.* 72:1155-1162 (1994).
4. Mayer I., McLean E., Kieffer T.J., Souza L.M., and Donaldson E.M. Antisomatostatin-induced growth acceleration in Chinook salmon (*Oncorhynchus tshawytscha*). *Fish Physiol. Biochem.* 13:295-300 (1994).
5. Kieffer T.J., McIntosh C.H.S., and Pederson R.A. Degradation of glucose-dependent insulintropic polypeptide (GIP) and truncated glucagon-like peptide-1 (tGLP-1) *in vitro* and *in vivo* by dipeptidyl peptidase IV. *Endocrinology* 136:3585-3596 (1995).
6. Kieffer T.J., Huang Z., McIntosh C.H.S., Buchan A.M.J., Brown J.C., and Pederson R.A. Gastric inhibitory polypeptide (GIP) release from a tumor-derived cell line (STC6-14). *Am. J. Physiol.* 269:E316-E322 (1995).
7. Morrow G.W., Kieffer T.J., McIntosh C.H.S., MacGillivray R.T.A., Brown J.C., St. Pierre S., and Pederson R.A. The insulintropic region of gastric inhibitory polypeptide; fragment analysis suggests the bioactive site lies between residues 19 and 30. *Can. J. Physiol. Pharmacol.* 74:65-72 (1996).
8. Pederson R.A., Kieffer T.J., Pauly R., Kofod H., Kwong J., and McIntosh C.H.S. The enteroinsular axis in dipeptidyl peptidase IV (DPPIV) negative rats. *Metabolism* 45:1335-1341 (1996).
9. Schieldrop P.J., Gelling R.W., Elliot R., Hewitt J., Kieffer T.J., McIntosh C.H.S., and Pederson R.A. Isolation of a murine glucose-dependent insulintropic polypeptide (GIP) cDNA from a tumor cell line (STC6-14) and quantification of glucose induced increases in GIP mRNA. *Biochem. Biophys. Acta.* 1308:111-113 (1996).

10. Heller R.S., Kieffer T.J. and Habener J.F. Point mutations in the first and third intracellular loops of the glucagon like peptide-1 receptor alter intracellular signaling. *Biochem. Biophys. Res. Commun.* 223:624-32 (1996).
11. Kieffer T.J., Heller R.S., and Habener J.F. Leptin receptors expressed on pancreatic β -cells. *Biochem. Biophys. Res. Commun.* 224:522-527 (1996).
12. Tseng C.C., Kieffer T.J., Jarboe L.A., Usdin T.B., and Wolfe M.M. Postprandial stimulation of insulin release by glucose-dependent insulinotropic polypeptide (GIP): effect of a specific GIP receptor antagonist. *J. Clin. Invest.* 98:2440-2445 (1996).
13. Kieffer T.J., Heller R.S., Unson C.C., Weir G.C., and Habener J.F. Distribution of glucagon receptors on hormone-specific endocrine cells of rat pancreatic islets. *Endocrinology* 137:5119-5125 (1996).
14. Heller R.S., Kieffer T.J., and Habener J.F. Insulinotropic glucagon-like peptide-1 receptor expression in glucagon-producing α -cells of the rat endocrine pancreas. *Diabetes* 46:785-791 (1997).
15. Kieffer T.J., Heller R.S., Leech C.A., Holz G.G., and Habener J.F. Leptin suppression of insulin secretion by the activation of ATP-sensitive K^+ channels in pancreatic β -cells. *Diabetes* 46:1087-1093 (1997).
16. Yip R.G.-C., Boylan M.O., Kieffer T.J., and Wolfe M.M. Functional GIP receptors are present on adipocytes. *Endocrinology* 139:4004-4007 (1998).
17. Seufert J.R., Kieffer T.J., Leech C.A., Holz G.G., Moritz W., Ricordi C., and Habener J.F. Leptin suppression of insulin secretion and gene expression in human pancreatic islets: implications for the development of adipogenic diabetes mellitus. *J. Clin. Endo. Metab.* 84:670-676 (1999).
18. Seufert J.R.*, Kieffer T.J.*, and Habener J.F. Leptin inhibits insulin gene transcription and reverses hyperinsulinemia in leptin-deficient *ob/ob* mice. *Proc. Natl. Acad. Sci. USA* 96:674-679 (1999). *Contributed equally to the studies.
19. Kieffer T.J., and Habener J.F. The glucagon-like peptides. *Endocr. Rev.* 20:1-38 (1999).
20. Kieffer T.J., and Habener J.F. The adipoinsular axis: effects of leptin on pancreatic beta-cells. *Am. J. Physiol.* 278:E1-E14 (2000).
21. Kieffer T.J., Lam N.T., and Cheung A.T. Insulin production from the β cell: antagonistic roles of GLP-1 and leptin. *Can. J. Diabetes Care* 24:47-57 (2000).

22. Stoffers D.A., Kieffer T.J., Hussain M.A., Drucker D.J., Bonner-Weir S., Habener J.F., and Egan J.M. Insulinotropic glucagon-like peptide-1 agonists stimulate expression of homeodomain protein IDX-1 and increase islet size in mouse pancreas. *Diabetes* 49:741-748 (2000).
23. Lewis J.T., Dayanandan B., Habener J.F., and Kieffer T.J. Glucose-dependent insulinotropic polypeptide confers early phase insulin release to oral glucose in rats: demonstration by a receptor antagonist. *Endocrinology* 141:3710-3716 (2000).
24. Baggio L., Kieffer T.J., and Drucker, D.J. GLP-1 but not GIP regulates fasting glycemia and non-enteral glucose clearance in mice. *Endocrinology* 141:3703-3709 (2000).
25. Cheung A.T., Dayanandan B., Lewis J.T., Korbitt G.S., Rajotte R.V., Boylan M.O., Wolfe M.M., and Kieffer T.J. Glucose-dependent insulin release from genetically engineered K cells. *Science* 290:1959-1962 (2000).
26. D'Alessio D., Kieffer T.J., Taborsky Jr. G.J., and Havel P.J. The parasympathetic nervous system is an essential mediator of post-absorptive insulin secretion in rhesus macaques. *J. Clin. Endo. Metab.* 86:1253-1259 (2001).
27. Lynn F.C., Pamir N., Ng E.H.C., McIntosh C.H.S., Kieffer T.J., and Pederson R.A. Defective glucose-dependent insulinotropic polypeptide receptor expression in diabetic fatty Zucker rats. *Diabetes* 50:1004-1111 (2001).
28. Daniel P.B., Kieffer T.J., Leech C.A. and Habener J.F. Novel alternatively spliced exon in the extracellular ligand-binding domain of the PACAP type 1 receptor (PAC1R) selectively increases ligand affinity and alters signal transduction coupling during spermatogenesis. *J. Biol. Chem.* 276:12938-12944 (2001).
29. Lam N.T., and Kieffer T.J. The multifaceted potential of glucagon-like peptide-1 as a therapeutic agent. *Minerva Endocrinol.* 27:79-93 (2002).
30. Cheung A.T., Woo S.L.C., and Kieffer T.J. Tissue Engineering Towards a Cure for Diabetes. (submitted).
31. Cheung A.T., Lewis J.T., Dayanandan B., Boylan M.O., Wolfe M., and Kieffer T.J. Meal-regulated insulin production from gut K-cells - putative targets for insulin gene transfer to treat type-1 diabetes. (submitted)
32. Lam N.T., Lewis J.T., Cheung A.T., Luk L.T., Wang J., Kolls J.K., and Kieffer T.J. Leptin increases hepatic insulin sensitivity and protein tyrosine phosphatase-1B expression. (submitted)

Book Chapters:

1. Wolfe M.M., Boylan M.O., Kieffer T.J., and Tseng C.-C. Glucose-dependent insulinotropic polypeptide (GIP): Incretin vs. enterogastrone. In: *Gastrointestinal Endocrinology*, M.P. Conn and G.H. Greeley (eds.), Humana Press, Totowa, NJ pp. 439-466 (1999).
2. Kieffer T.J., Hussain M.A., and Habener J.F. Glucagon and glucagon-like peptide production and degradation. In: *Handbook of Physiology, Section 7: The Endocrine System, Vol 2: The Endocrine Pancreas and Regulation of Metabolism*, L.S. Jefferson and A.D. Cherrington (eds.), Oxford University Press, New York, NY pp197-265 (2001).
3. Habener J.F., and Kieffer T.J. Glucagon and glucagon-like peptides. In: *Joslin's Textbook of Diabetes Mellitus, 14th Edition*. R.C. Kahn, G.C. Weir, G.L. King, A.C. Moses, R.J. Smith, A.M. Jacobson (eds.), Joslin Diabetes Center, Boston, MA (in press).
4. Kieffer T.J. Glucose-dependent insulinotropic polypeptide (GIP): Role in energy homeostasis. In: *Encyclopedia of Hormones & Related Cell Regulators*, G.H. Greeley (ed), Academic Press, New York, NY (submitted).

Abstracts (1996-2001):

1. Heller R.S., Kieffer T.J., and Habener J.F. Immunolocalization of the glucagon-like peptide-1 receptor on all β and δ and selected α cells in rat pancreatic islets. *Endo. Soc.* 413 (1996).
2. Kieffer T.J., Heller R.S., and Habener J.F. Characteristics of glucagon-like peptide-1 (GLP-1) binding sites on 3T3-L1 adipocytes. *Endo. Soc.* 412 (1996).
3. Tseng C.C., Kieffer T.J., Jarboe L.A., Usdin T.B., and Wolfe M.M. Postprandial stimulation of insulin release by glucose-dependent insulinotropic polypeptide (GIP): effect of a specific GIP receptor antagonist. *Endo. Soc.* 97 (1996).
4. Heller R.S., Kieffer T.J., and Habener J.F. Single amino acid substitutions in the first and third intracellular loops of the glucagon-like peptide-1 receptor impair intracellular signaling. *Diabetes*, 45 Suppl 2: 117A (1996).
5. Kieffer T.J., Heller R.S., and Habener J.F. Glucagon receptors on pancreatic β -cells. *Regul. Pept.* 50:21 (1996).

6. Kieffer T.J., Heller R.S., and Habener J.F. Distribution of leptin receptors on pancreatic islets. *Diabetes*, 46 Suppl 1: 80A (1997).
7. Kieffer T.J., and Habner J.F. Development of a GIP receptor antagonist. *Diabetes*, 47 Suppl 1: A192 (1998).
8. Daniel P.B., Kieffer T.J., Leech C.A., and Habener J.F. A novel type 1 PACAP receptor splice variant in rat testis. *Endo. Soc.* 270 (1998).
9. Kieffer T.J., Heller R.S., Leech C.A., Holz G.G., and Habener J.F. Leptin inhibits insulin gene expression and secretion. *CFBS 41st* 248 (1998).
10. Seufert J., Kieffer T.J., and Habener J.F. Leptin inhibits insulin gene transcription and reverses hyperinsulinemia in leptin-deficient *ob/ob* mice. *Endo. Soc.* 113-114 (1999).
11. Seufert J., Kieffer T.J., Leech C.A., Holz G.G., Moritz W., Ricordi C., and Habener J.F. Leptin suppression of insulin secretion and gene expression in human pancreatic islets: implications for the development of adipogenic diabetes mellitus. *Diabetes*, 48 Suppl 1: A235 (1999).
12. Kieffer T.J. Insulin secretion from the beta cell: role of receptors and channels. *CDA/CSEM Proceedings*, P46 Ottawa (1999).
13. Lam N.T., Cheeseman C., and Kieffer T.J. Leptin alters glucose transport in hepatocytes. *CDA/CSEM Proceedings*, A43 Ottawa (1999).
14. Korbitt G.S., Rayat G.R., Rajotte R.V., and Kieffer T.J. Culture of microencapsulated neonatal porcine islets is associated with an increased proportion of β cells/cellular insulin content and elevated expression of IDX-1. *Diabetes*, 48 Suppl 1: A474 (1999).
15. Korbitt G.S., Rayat G.R., Rajotte R.V., and Kieffer T.J. In vitro expansion of neonatal porcine islet cells is associated with elevated expression of IDX-1 and NKX 2.2. *Diabetologia*, 42 Suppl 1: A151 (1999).
16. Chelikani P.K., Ambrose J.D., Glimm D.R., Kieffer T.J., and Kennelly J.J. Plasma leptin concentrations in dairy cows: I) Effect of short-term fasting and refeeding. *J. Anim. Sci.*, 78 Suppl 1: A859 (2000)
17. Chelikani P.K., Ambrose J.D., Glimm D.R., Kieffer T.J., and Kennelly J.J. Plasma leptin concentrations in dairy cows: II) Effect of feeding or postruminal infusion of canola oil. *J. Anim. Sci.*, 78 Suppl 1: A74 (2000)

18. Korbitt G.S., Rayat G.R., Binette T.M., Rajotte R.V., and Kieffer T.J. Development of an *in vitro* model for islet cell neogenesis. *Stem Cells and Pancreatic Development*, Bethesda MD (2000).
19. Cheung A.T., Dayanandan B., Lewis J.T., Korbitt G.S., Rajotte R.V., Boylan M.O., Wolfe M.M., and Kieffer T.J. A novel gene therapy target for diabetes: expression of insulin in gut K-cells. *American Diabetes Association Presidential Poster Session*, San Antonio TX (2000).
20. Baggio L., Kieffer T.J., and Drucker, D.J. Incretin antagonists define contrasting roles for GLP-1 and GIP in the control of glucose homeostasis in vivo. *Endo. Soc. 82nd Annual Meeting*, A291 (2000).
21. Stoffers D.A., Kieffer T.J., Hussain M.A., Drucker D.J., Bonner-Weir S., Habener J.F., and Egan J.M. Insulinotropic glucagon-like peptide-1 agonists stimulate expression of homeodomain protein IDX-1 and increase islet size in mouse pancreas. *Diabetes*, 49 Suppl 1: A179 (2000).
22. Lam N.T., Cheung A.T., and Kieffer T.J. Leptin reduces glucose transport and GLUT2 phosphorylation in beta-cells. *CDA/CSEM Proceedings*, A139 Halifax (2000).
23. Seufert J.R., Laubner K., Royer S., Kieffer T.J., and Jakob F.J. Proinsulin gene transcription activation by synergistic interactions of STAT5B and the homeodomain protein IDX-1 with the CBP/P300 coactivator in pancreatic beta-cells. *Endo. Soc. 83rd Annual Meeting*, OR13-6 (2001).
24. Cheung A.T., Lewis J.T., Dayanandan B., and Kieffer T.J. Meal-regulated insulin production from intestinal K-cells. *Diabetes*, 50 Suppl 2: A6 (2001).
25. Lam N.T., Luk C.T., Lewis J.T., Cheung A.T., and Kieffer T.J. Leptin acutely increases hepatic insulin sensitivity and PTIP-1B levels. *Diabetes*, 50 Suppl 2: A369 (2001).
26. Lynn F.C., Pamir N., Ng E.H.C., McIntosh C.H.S., Kieffer T.J., and Pederson R.A. Defective glucose-dependent insulinotropic polypeptide receptor expression in diabetic fatty Zucker rats. *Diabetes*, 50 Suppl 2: A73 (2001).
27. Seufert J.R., Laubner K., Kieffer T.J., and Jakob F.J. Leptin mediated signal transduction in pancreatic beta-cells. *Diabetes*, 50 Suppl 2: A341 (2001).
28. Seufert J.R., Kieffer T.J., and Jakob F.J. Wortmannin sensitive nuclear translocation and gene regulation by the transcription factor PDX-1 mediates glucose dependent activation of the rat insulin-1 promoter by glucagon-like peptide 1 (GLP-1). *Diabetes*, 50 Suppl 2: A346 (2001).

OTHER PROFESSIONAL AND SCHOLARLY ACTIVITIES

Major Committee Assignments:

- 
- | | |
|-------------|---|
| 1995 - 1996 | Clinical Research Data Search Engine Design
Massachusetts General Hospital |
| 1999 - 2001 | Biological Sciences Technology Laboratory and Research Program
Advisory Committee
The Northern Alberta Institute of Technology (NAIT) |
| 2000 - 2002 | American Diabetes Association 61 st Scientific Sessions Planning
Committee, Subcommittee on Hormones, not Insulin
Development of 2001 Sessions Program and Abstract Reviewer |
| 2000 - 2003 | Canadian Institutes of Health Research Grants Committee Member
Metabolism and Nutrition |
| 2001 - 2002 | Canadian Diabetes Association Grants Committee Member
Committee B |
| 2001 - 2004 | Juvenile Diabetes Research Foundation Grants Committee Member
Medical Science Review Committee |
| 2001 - 2002 | Research Committee of the UA Department of Medicine |

Professional Society Memberships:

- | | |
|------|---|
| 1995 | Endocrine Society |
| 1995 | American Diabetes Association |
| 1996 | American Physiological Society |
| 1996 | Canadian Physiological Society |
| 1997 | Canadian Diabetes Association |
| 1997 | Canadian Federation of Biological Societies |

Institutional Memberships:

- | | |
|------|---|
| 1998 | Muttart Diabetes Research and Training Centre |
|------|---|

Editorial Boards:

- | | |
|------|---|
| 2001 | <i>e-biomed: The Journal of Regenerative Medicine</i>
Published by Mary Ann Liebert Inc. |
|------|---|

Conference Chair:

- | | |
|------|---|
| 2002 | American Diabetes Association 62nd Scientific Sessions
Session: <i>GLP-1 and GIP</i> |
|------|---|



FIGURE 1: NORTHERN BLOT AND GRAPH (*in-vitro* study)

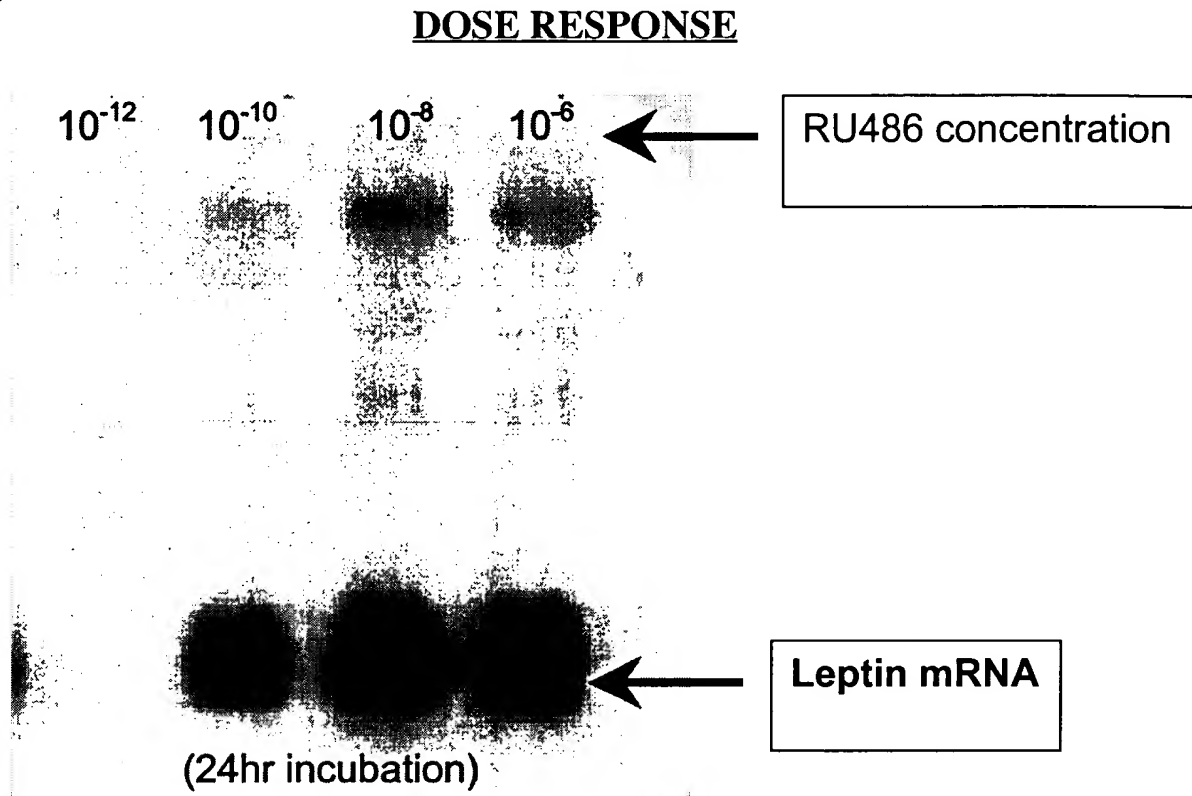
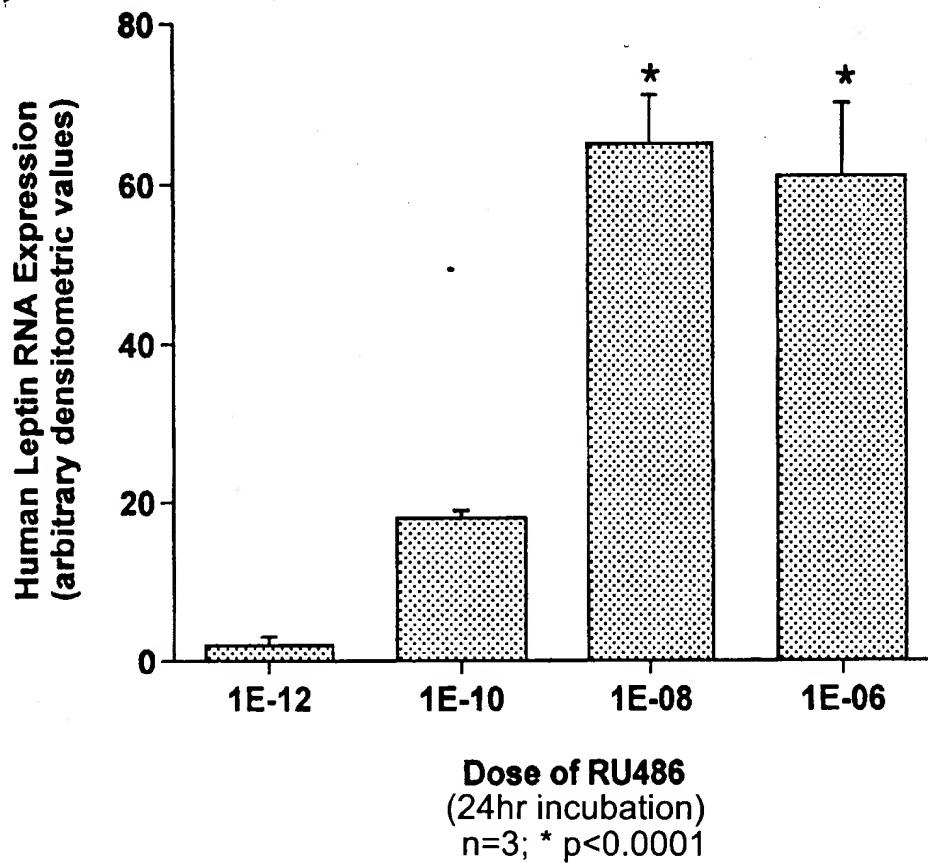


FIGURE 2: NORTHERN BLOT AND GRAPH (*in-vitro* study)

DOSE RESPONSE

**Dose Response for Leptin RNA Expression in
pSwitch/GIPro/hLeptin cells with RU486 Treatment**



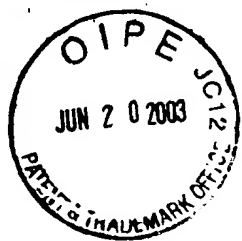


FIGURE 3: NORTHERN BLOT AND GRAPH (*in-vitro* study)

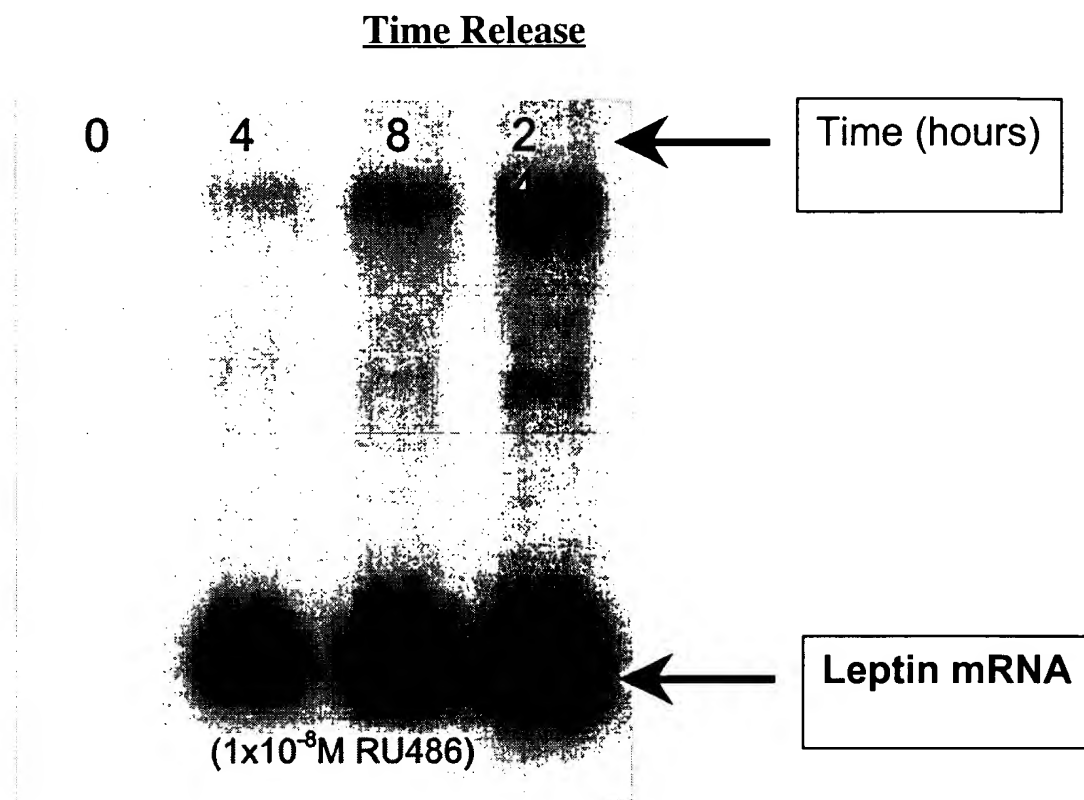




FIGURE 4: NORTHERN BLOT AND GRAPH (*in-vitro* study)

Time Release

**Time Course for Leptin RNA Expression in
pSwitch/GIPro/hLeptin cells with RU486**
Treatment

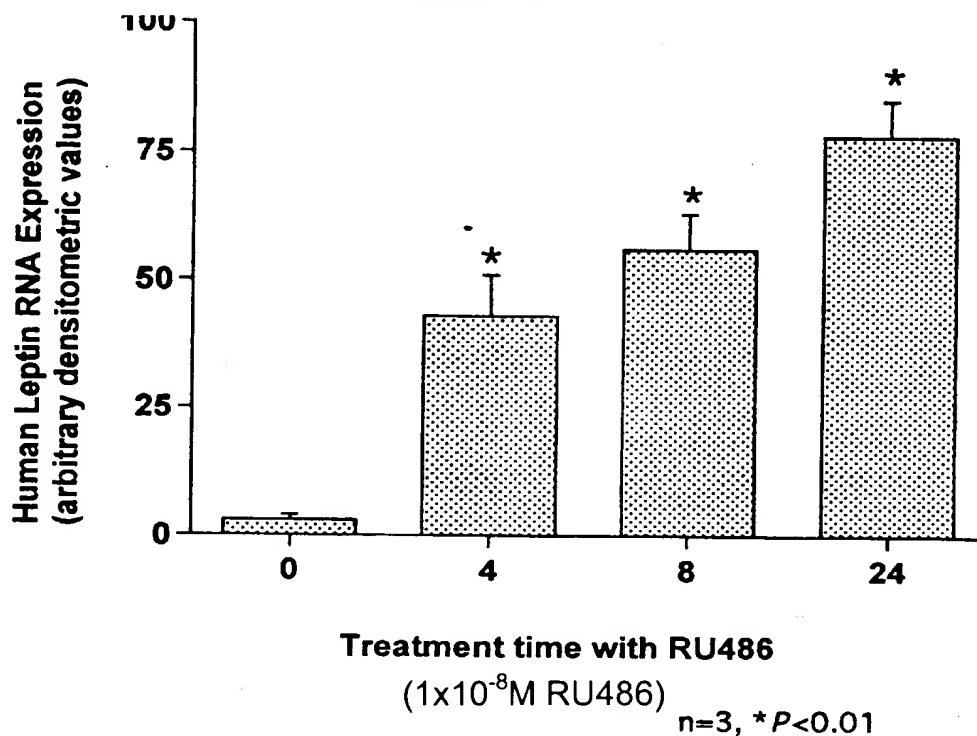




FIGURE 5: WESTERN BLOT DATA (*in-vitro* study)

DOSE RESPONSE

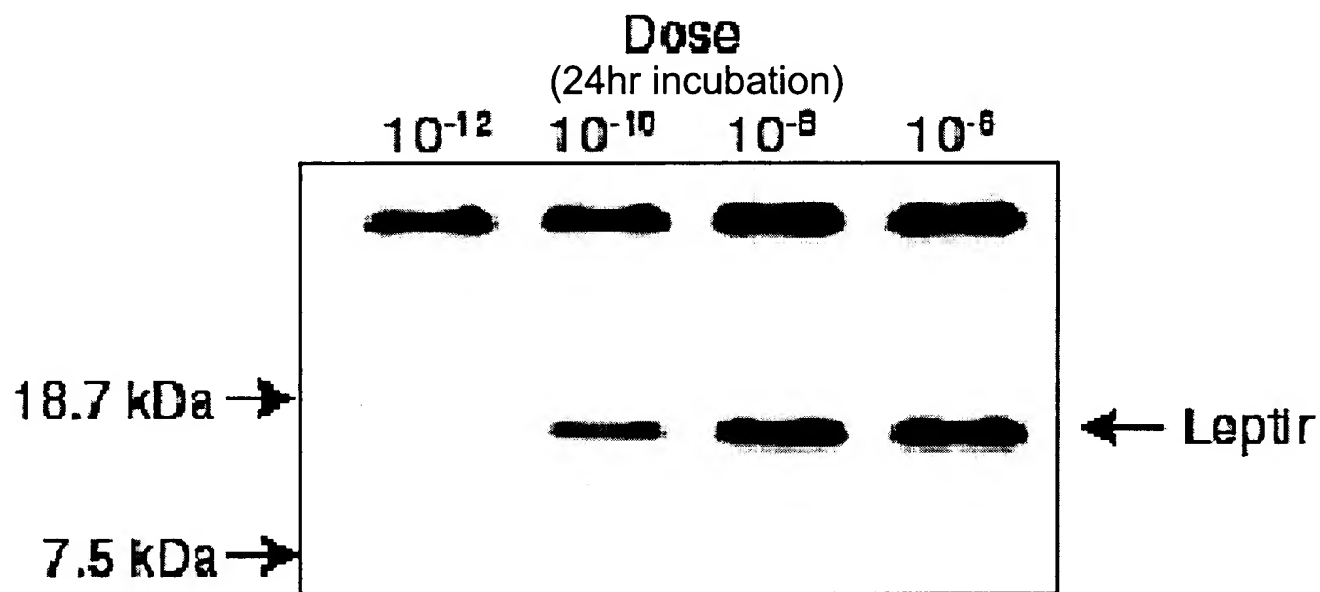
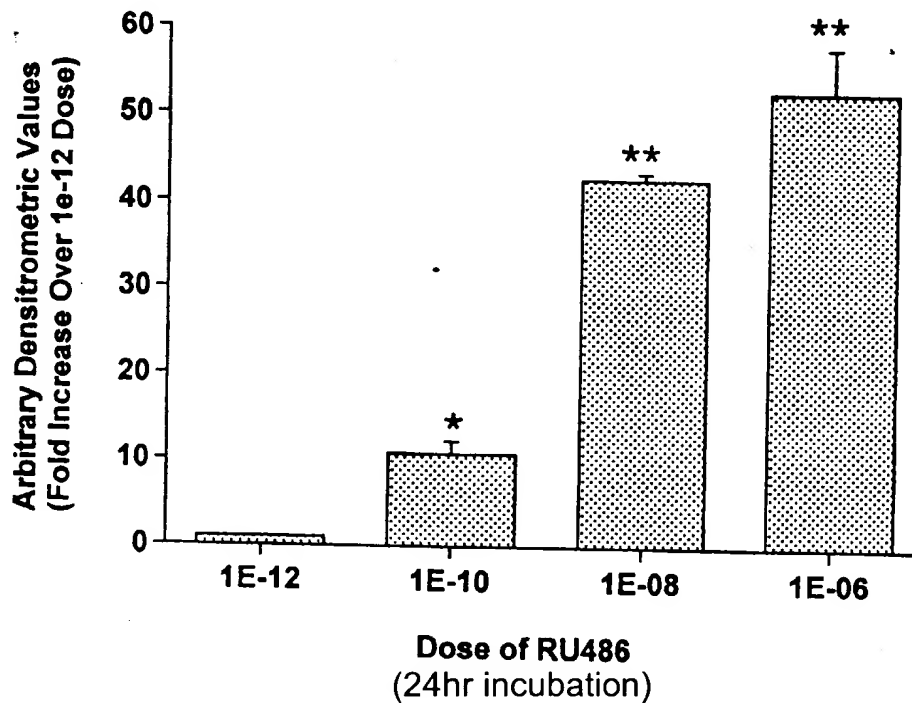




FIGURE 6: WESTERN BLOT DATA (*in-vitro* study)

Dose Response for Human Leptin Protein Expression



n=3, * $P < 0.05$, ** $P < 0.001$ compared to 1e-12 dose



FIGURE 7: WESTERN BLOT DATA (*in-vitro* study)

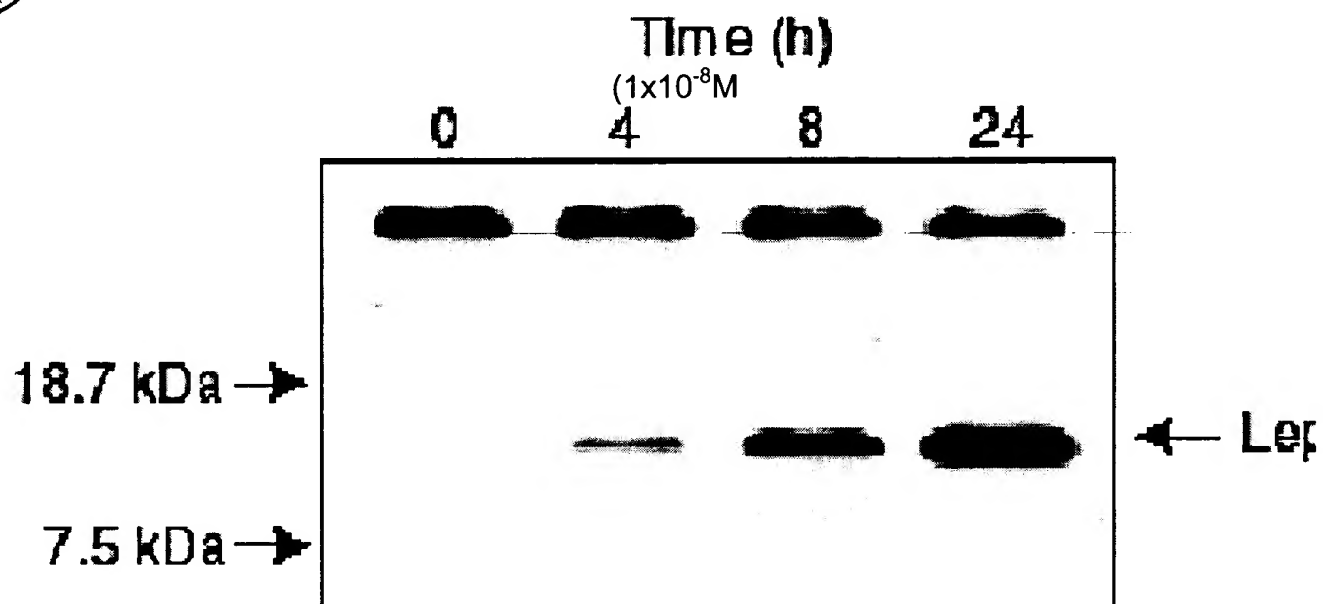
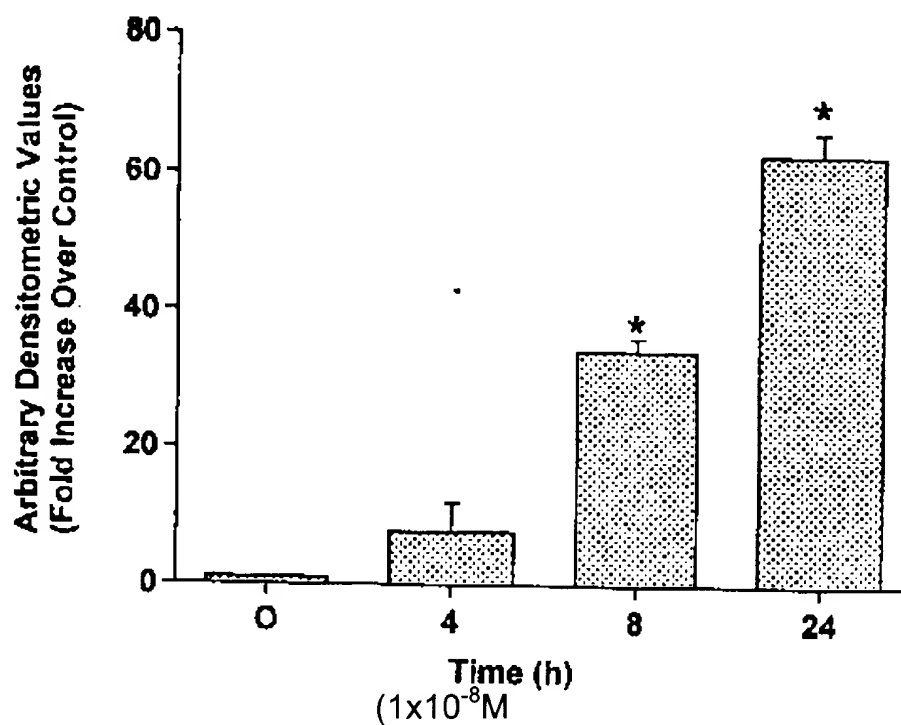




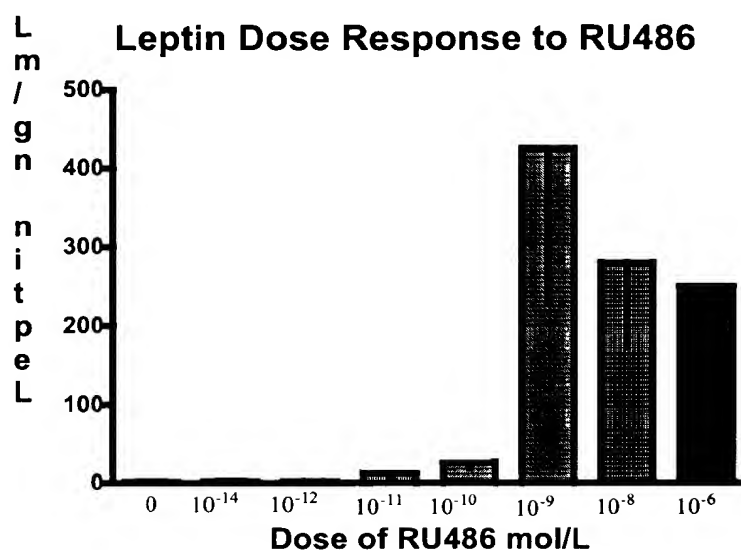
FIGURE 8: WESTERN BLOT DATA (*in-vitro* study)

Time Course for Human Leptin Protein Expression



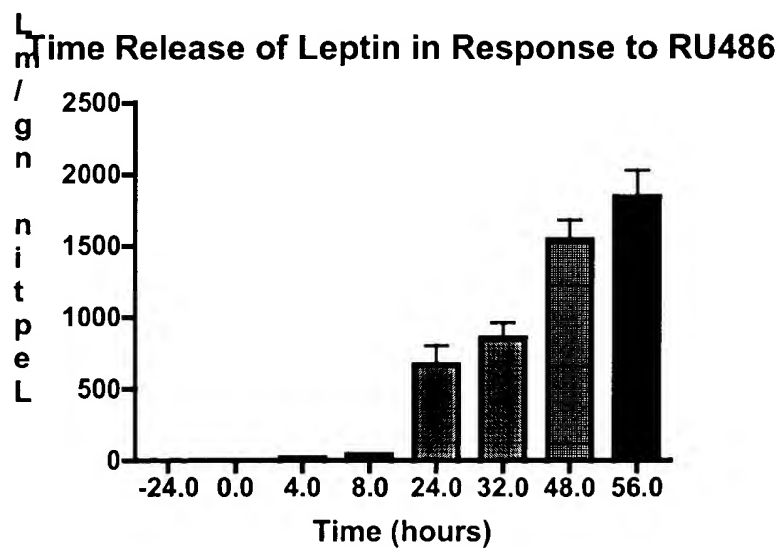
n=3, * $P < 0.001$ compared to Time 0

FIGURE 9A: ENZYME LINKED IMMUNOSORBENT ASSAY (*in-vitro* study)



n=6; treatment duration = 24hours
* $p < 0.05$

FIGURE 9B: ENZYME LINKED IMMUNOSORBENT ASSAY (*in-vitro* study)



n=6; dose used = 1×10^{-8} M RU486



FIGURE 10: *in-vivo* study

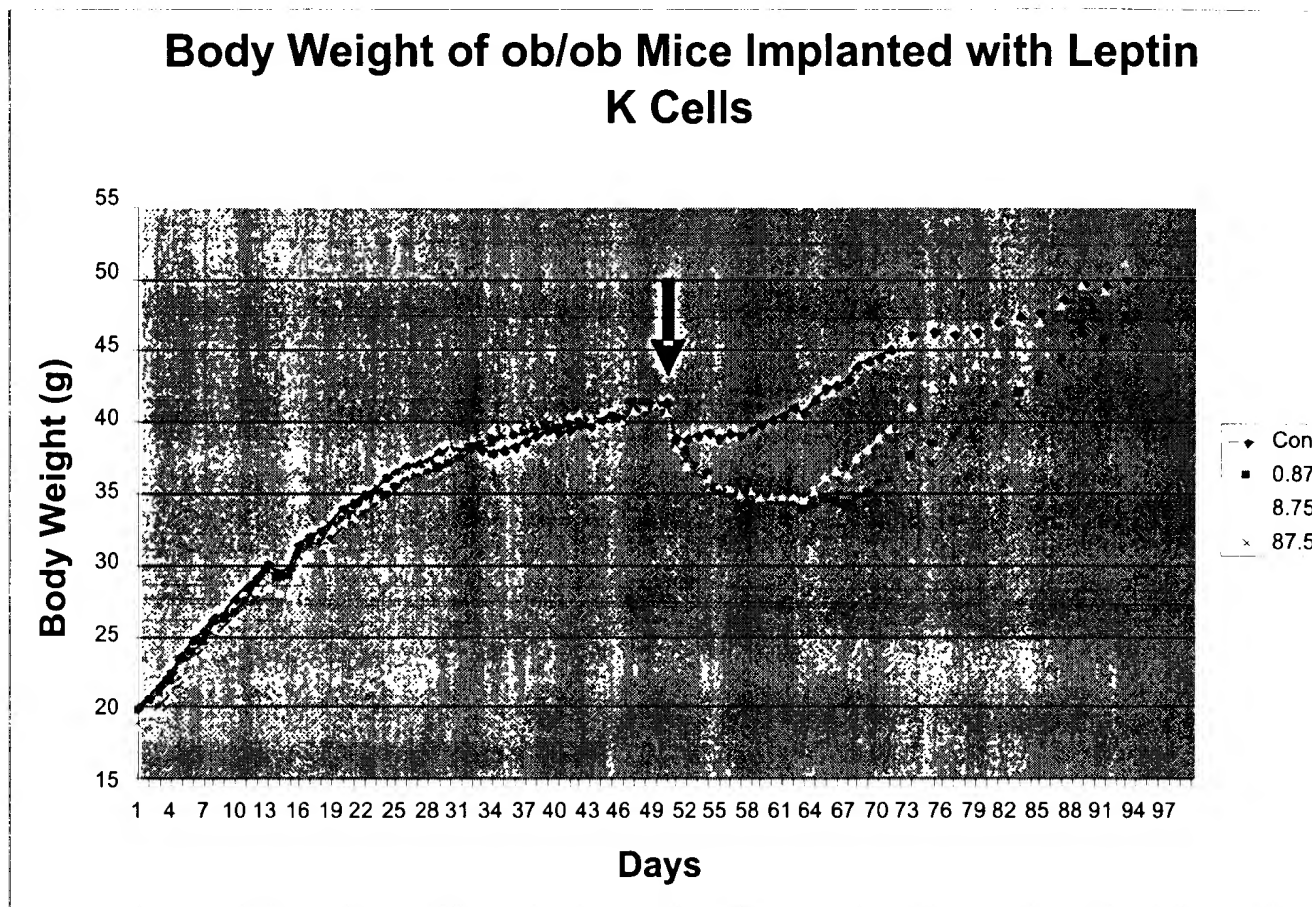




FIGURE 11: *in-vivo* study

Blood Glucose Concentrations of Ob/Ob Mice Implanted with Leptin-Producing K Cells

